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Testicular morphology in Corriedale rams

Influence of feeding management under extensive rearing conditions in the Río de la Plata grasslands

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To love for everyone and for everything

To my wife and daughters

Abstract

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Seasonality is a basic trait of sheep reproduction. Nutrition and photoperiod interact to modulate the seasonality of ram testis form and function. The influences of these cues vary according to breed, location, and management strategies applied. Since nutrition affects testis development, feeding conditions during early life might influence testis activity later. The overall aim of this thesis was to study the influence of seasonality and feeding regimes on testis morphometry in Corriedale rams reared on extensive systems in Uruguay. Experimental studies looked for influences of differential feeding management on testis morphology and function, particularly on seasonal changes and on testis development, from foetal to post-pubertal life.

Testis morphology was studied in 24 rams grazing either improved or native pastures over a one-year period. Clinical recordings, testosterone measurements, and quantitative histology of testes, epididymides, and seminal vesicles were performed in autumn (March), winter (July), spring (September), and summer (December). Both groups had highest values in autumn, lowest in winter, and recovered thereafter. Rams grazing improved pastures showed a somewhat smaller testicular winter regression and recovered earlier in spring.

The influence of improved feeding on the seasonal (autumn-winter) changes in testicular form and function was studied in rams fed improved pastures plus grain supplement (controls were grazing native pasture). Castration/histology studies were performed in autumn (March: autumn control group, $n = 6$, native pasture) and in winter (August: winter control group, $n = 7$, native pasture; treated group, $n = 7$, improved pasture plus grain supplement). Clinical recordings and testosterone measurements were performed monthly. The seasonal testis regression was described morphometrically. The treatment applied alleviated slightly this regression, but did not reverse the declining trend in testicular size and activity.

The influence of long-lasting differential grazing regimes from 1 to 30 months of age on testicular growth and morphology by adult age was studied in 41 ram lambs. Animals grazed either native or improved pastures (groups H and L, respectively; 1–8 months of age, Period 1). By moving half the animals to the opposite treatment, groups HH, HL, LH, and LL were created (8–18 months of age, Period 2). Finally, all remaining rams shared the same grazing regime (18–30 months of age, Period 3). Clinical results were recorded every other month. Half the animals from each group were castrated for histology at 18 and 30 months of age. Scrotal circumference (SC) was higher in group H during Period 1. During Period 2, the SC of group LH rams equalized values of group HH, and SC in groups HL and LH reached similar values. Testis weight, volume, and stereological parameters did not differ between groups by the end of Periods 2 or 3. No differences in SC were seen between groups during most of Period 3.

The impact of improved feeding of the ewe-lamb unit during foetal and early post-natal life on the testicular development of the ram lamb was studied in 14 ewe-lamb units fed improved pasture plus grain supplement from mid-pregnancy to pre-pubertal life, as compared with 12 ewe-lamb units grazing native pasture (half of lambs castrated for histology at 99 days of age). Clinical and endocrinological studies were performed from birth to castration. Puberty was hastened in grain-supplemented lambs and their Sertoli cell numbers tended to be higher.

In conclusion, this thesis describes the morphometry of the seasonal testis regression of Corriedale rams and shows that this regression is not easily reverted by the hereby tested level of improved feeding. The enhancement in testis development when pre- and post-pubertal Corriedale rams grazed improved pasture was short-lived. The tendency to higher Sertoli cell numbers elicited by treating animals with improved pasture plus grain supplementation from foetal to pre-pubertal life might result in longer-lived consequences in spermatogenetic capacity.

Key words: Corriedale, ram, lamb, season, nutrition, pastures, stereology, spermatogenesis, Sertoli cell numbers, scrotal circumference, testosterone, FSH.

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Appendix

Papers I-V

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals **I-V**:

I. Bielli A, Gastel T, Pérez, R, López A, Castrillejo A, Regueiro M, Forsberg M, Lundeheim N and Rodriguez-Martinez H. Influence of nutrition on seasonal variations in testicular morphology and function in Corriedale rams. *J Reprod Dev 1997; 43:171-180.*

II. Bielli A, Pedrana G, Gastel MT, Castrillejo A, Moraña A, Lundeheim N, Forsberg M and Rodriguez-Martinez H. Influence of grazing management on the seasonal change in testicular morphology in Corriedale rams. *Anim Reprod Sci, 1999 (in press).*

III. Bielli A, Gastel MT, Pedrana G, Moraña A, Castrillejo A, Lundeheim N, Forsberg M and Rodriguez-Martinez H. Influence of pre- and post-pubertal grazing regimes on adult testicular morphology in extensively reared Corriedale rams. *(submitted).*

IV. Bielli A, Katz H, Pedrana G, Gastel MT, Moraña A, Castrillejo A, Lundeheim N, Forsberg M and Rodriguez-Martinez H. Grazing management during foetal and post-natal life influences testicular stereology in Corriedale ram lambs. *(submitted).*

General Introduction

Studies of testicular morphology and activity of a particular sheep breed require background information regarding sheep reproductive strategies, testicular histology and characteristics of the rearing system applied locally. Consequently, a summarised review on these subjects concerning Corriedale sheep in the Río de la Plata grassland region is presented below.

Seasonality in form and function of the ram testes

An animal's evolutionary success depends on its capacity to pass on its genetic make-up to the next generation and hence, on such an animal's capacity to produce as many offspring as possible that reach reproductive age (Strickberger, 1990). To maximise the chances of survival of both mother and offspring, animals must find strategies to time their reproductive activities so that late pregnancy and lactation (the periods that are most costly in energy for the mother, and when the newborn is most vulnerable) occur during the most benign part of the year. In order to warrant this timing properly, environmental cues allowing animals to foresee seasonal variations in environment, such as photoperiod, nutrition, temperature and social factors regulate their breeding cycle. Sheep breeds originated from higher latitudes time their reproductive cycle according to annual variations in photoperiod length (Lincoln and Short, 1980), so that births occur in spring. In the ram, seasonal changes can be noticed by photoperiod-driven cycles in testosterone and FSH secretion, testis size, efficiency of spermatogenesis, sperm quality, ejaculate volume and mating activity, with all these parameters peaking in autumn (Amir and Volcani, 1965; Schanbacher and Ford, 1976; Sanford et al., 1977). The magnitude of photoperiod variation between the shortest and longest day in a given location influences the degree of seasonality expressed by sheep at that place. Moving ewes from higher to lower latitudes extends the duration of their breeding season (Hulet et al., 1974) and it has been noticed that rams of the same breed reared nearer the equator experience smaller annual variations in testis size (Setchell, 1992). Genetic influences on seasonal reproduction are also very important in sheep. Breeds originated from lower latitudes tend to have earlier and longer "breeding periods", with lower amplitude in seasonal variations of reproductive parameters (Lincoln et al., 1990). As photoperiodic cueing becomes weaker, other environmental cues, such as nutrition, become more important. The Merino, having originated in a climate where pasture growth, and hence food, is linked to rainfall, have evolved a reproductive strategy of weak photoresponsiveness and strong responsiveness to nutrition. Under field conditions, in Mediterranean-like climates where feeding is better in winter and spring, Merino rams attain maximum testis size in early summer and by autumn their testes are already regressing (Masters and Fels, 1984). Changes in testis size due to changes in nutrition are very consistent in Merino rams, and nutrition improvement in any season provokes an increase of their testis size (Murray et

al., 1991). Nutritional influences on testis size are evidenced in very seasonal sheep breeds (i.e. Soay) when photoperiodic cueing is eliminated surgically (Lincoln et al., 1989).

Puberty and nutrition in rams

Puberty can be defined as the onset of fertility and the period of rapid development that precedes it (Bronson and Rissman, 1986). During this period, the GnRH neurosecretory system is modulated by cues relating physiologic body size and well being. When the metabolic signals indicate inadequate animal size, the activity of the GnRH system, and hence LH secretion, are low. When adequate body size is reached, the activity in the GnRH neurosecretory system increases. The pubertal period is marked then by an increase in LH and FSH secretion. One of the main factors in the environmental regulation of puberty in mammals is, therefore, the access to adequate nutrition (Bronson and Rissman, 1986). The ram lamb is no exception. Nutritional restrictions before puberty delay lambs testicular growth, and their rate of sexual development is highly dependent on food energy intake and live weight gain, both in highly seasonal (Dýrmundsson, 1973) and less seasonal (Dunn, 1955) sheep breeds. Furthermore, there is considerable variation between and within breeds in the rate of post-natal testicular development (Dýrmundsson, 1973; Lafortune et al., 1984; Osinowo et al., 1992).

Testis histology, Sertoli cells and spermatogenesis

Testis histology

The testis of an adult ram weighs normally 150-300 g. It is covered by the tunica albuginea, a fibrous capsule sending strands into the parenchyma. These strands merge in the centre of the testis to form an axial sector, the mediastinum testis where the straight tubules, rete testis and efferent ducts, building the excretory duct system of the testis, are located (Amman, 1970). Most of the testicular parenchyma is of seminiferous nature. The seminiferous parenchyma is composed by the convoluted seminiferous tubules and the testicular interstitium. The testicular interstitium harbours interstitial Leydig cells, loose connective tissue cells, blood vessels, lymphatics, and nerves. In the ram, the connective tissue is abundant, and Leydig cells either gather around blood vessels or cluster farther away from them (Setchell, 1978). Most of the seminiferous parenchyma (approximately 85% in rams) is occupied by the seminiferous tubules (Wrobel et al., 1995). These are highly coiled tubes containing the seminiferous epithelium. Boundary tissue surrounds the tubules, being composed of an inner non-cellular layer, some myoid or smooth muscle-like cells, and some outer fibroblast-like cells and collagen-like fibres (Setchell, 1978).

The seminiferous epithelium contains the germinal cells and the somatic Sertoli cells. Sertoli cells extend from the boundary tissue to the lumen of the tubule. They are tree-like cells with numerous prolongations extending laterally and luminally (Russell, 1993). Sertoli cells have a complex, indented nucleus, with a

prominent nucleolus. Mitochondria, Golgi complexes and dense bodies are abundant. Rough endoplasmatic reticulum (ER) is abundant in the basal part of the cell, and smooth-ER is spread all over the cell, particularly near the elongating spermatids. The development and functional integrity of the seminiferous epithelium is largely dependent upon the Sertoli cells (Griswold, 1995). These cells determine the general structure of the germinal epithelium, mainly by way of intercellular junctions and cytoplasmic prolongations, surrounding germ cells and controlling their location and metabolism.

Spermatogenesis is a highly organised and precisely timed process, whereby diploid stem-cell spermatogonia produce haploid spermatozoa (Sharpe, 1994), through a process that lasts 49 days in the ram (Courot et al., 1970). Spermatogenesis can be divided into three phases: the proliferative phase, during which spermatogonia undergo several mitosis and finally give rise to preleptotenic primary spermatocytes; the meiotic phase, during which primary, tetraploid spermatocytes divide into secondary, diploid spermatocytes and further into round, haploid spermatids; and the spermiogenic phase, during which round spermatids differentiate into elongated spermatids to be finally released by the Sertoli cell as testicular spermatozoa into the tubular lumen (Russell et al., 1990).

Spermatogenesis is regulated at the local level by a complex interaction between Sertoli cells and specific associations of germ cells, which constitute the different stages of the seminiferous cycle (8 stages in the ram, Hochereau-de Reviers et al., 1990). Sertoli cells form two permanent compartments (basal and adluminal) within the seminiferous epithelium. The compartments are divided by cytoplasmic prolongations of adjacent Sertoli cells, linked by tight and occluding specialized junctions. The cells in the basal compartment include spermatogonia and spermatocytes up to the early leptotene phase of meiosis (Russell et al., 1990). Leptotene spermatocytes are transitorily surrounded by Sertoli cell prolongations both luminally and basally. Later on, there is a breakdown of the tight junctions linking the prolongations displayed luminally to leptotene spermatocytes, and these germ cells are integrated into the adluminal compartment. The junctional complexes separating the adluminal and basal compartments constitute the last, and major, component of the blood-testis barrier, which effectively excludes macromolecules from entering the adluminal compartment. The micro-environment in the adluminal part of the seminiferous epithelium is composed of secretions from Sertoli cells and germ cells (Griswold, 1995). Germ cells within the adluminal compartment depend on Sertoli cells for nutrients and metabolic regulation. Most germ cells seem to lack receptors for testosterone or FSH, but Sertoli cells do have these types of receptors, and are sensitive to the corresponding hormones (Griswold, 1995). Myoid and Leydig cells also participate in the local regulation of spermatogenesis. Numerous factors and metabolites, other than testosterone, are secreted by Leydig cells (i.e. interleukins –1 and –6, TGF beta, Caussanel et al.,

1997) and by myoid cells (i.e. P-Mod-S, TGF beta, IGF-I, activin-A, Maekawa et al., 1996). These factors are known to influence Sertoli cell activity in laboratory animals, boars and humans.

Both gonadotrophins and testosterone appear to be necessary to maintain spermatogenesis qualitatively and quantitatively. LH probably stimulates the differentiation of renewing spermatogonia (Courot et al., 1979; Courot and Ortavant, 1981). Testosterone appears to control the production of intermediate spermatogonia, the meiotic phase and spermiogenesis (Courot and Ortavant, 1981; Sharpe et al., 1990; Kilgour et al., 1993), and FSH seems to control the last spermatogonial divisions and primary spermatocyte production (Courot et al., 1979; Courot et al., 1984; Kilgour et al., 1993).

Short- and long-lived determinants of sperm output:

1. Sertoli cell numbers

Sertoli cell numbers are highly correlated with adult testis size. The number of Sertoli cells in the testes of an animal sets a ceiling to the maximal germ cell numbers such an animal can produce, thus limiting maximal sperm output capacity (for review see Sharpe, 1994). Maximal Sertoli cell numbers are determined prior to puberty. In fact, inhibition of Sertoli cell mitosis in newborn rats results in fewer Sertoli cells and hence reduced sperm output and testicular weight during adulthood (Orth et al., 1988). Furthermore, if the period during which Sertoli cells can divide is prolonged by inducing transient hypothyroidism, an increased number of Sertoli cells is obtained, and hence both adult testicular weight and sperm output increase as well (Cooke, 1991). On the other hand, treating gonadotrophin-deficient pre-pubertal mice with FSH increases their population of Sertoli cells (Singh and Handelsman, 1996). However, while newborn muridae are altricial, newborn lambs are precocial offspring. The differences in developmental strategies during pregnancy and neonatal life between both species, determine that results from either type of animals are not readily comparable.

2. Efficiency of spermatogenesis

Once Sertoli cell replication has ceased, the quantitative production of spermatozoa depends on the total number of stem spermatogonia per testis, the number of spermatogonial generations between stem cells and primary spermatocytes, the scheme of stem cell renewal and the yield of spermatogonial divisions (Courot et al., 1970). This last yield is relatively low in the ram (around 50%), and varies seasonally according to plasma levels of LH (Hochereau-de Reviers et al., 1976). Later on, the final number of elongated spermatids or testicular spermatozoa will also depend on germ cell degeneration during meiosis and during spermiogenesis. Ortavant (1956) suggested that loss of germ cells in rams exposed to long days occurred at all stages of spermatogenesis. Germ cell degeneration during spermiogenesis does exist, but its rate in adult

mammals is considered to be unimportant as compared to what occurs during meiosis. Germ cell degeneration during meiosis is very low in the ram during the breeding season, to become higher during the non-breeding season (Ortavant, 1956).

Histological aspects of puberty

During the pre-pubertal period, both Sertoli cells and early germ cells (pre-spermatogonia) are multiplying. When puberty begins, spermatogonia begin proliferation, presumably under the control of Sertoli cells. Adjacent Sertoli cells establish tight junctions linking each other and a lumen appears in the axis of the testicular sex cords, that turn into seminiferous tubules. The conditions exist then for meiosis to begin, and finally spermatozoa are formed. Initially, many germ cells degenerate, but later the yield improves. The increase in testis size linked to puberty is due mainly to an increase in the volume of the seminiferous epithelium, first due to Sertoli cells and pre-spermatogonial divisions, and then to the beginning and establishment of spermatogenesis (Courot, 1971).

The general organisation of spermatogenesis is essentially the same in all mammals, and it is likely that many of the key regulatory events are conserved between species (Sharpe, 1994). However, this does not imply that the regulation of spermatogenesis is identical in all mammals. Moreover, since the various species are adapted to different ecological niches and have particular body sizes and evolutionary histories, they will react very differently to environmental stimuli, i.e. photoperiod, nutrition, social cues, etc.

The Río de la Plata grasslands

The Río de la Plata grasslands (see Fig. 1), constitute a phytogeographical province of temperate to sub-tropical sub-humid grasslands extending as an arc around this South American estuary, and covering some 700,000 sq. km from central-eastern Argentina to Uruguay and southern Brazil (Soriano, 1992). In contrast with Argentina's flat "**pampas**", the grasslands of Uruguay and southern Brazil consist of rolling plains (the so-called "**campos**") and low hills extending over the southern fringe of the eroded Brazilian shield (Burkart, 1975). Streams and rivers are surrounded by gallery forests, with trees reaching at most 8 m height (Joly, 1970). Before European colonisation, the area was covered with tall prairie dominated by species of feather grass (*Stipa*) and melic (*Melica*) (Eyre, 1968). Shrubby vegetation dominated hilly areas. At present, most grasses are sub-tropical species that grow during spring, summer and autumn. A small number of temperate climate species grow during autumn and spring and for short periods in winter. Thus the availability of winter forage tends to be limited due to diminished solar radiation and temperature.

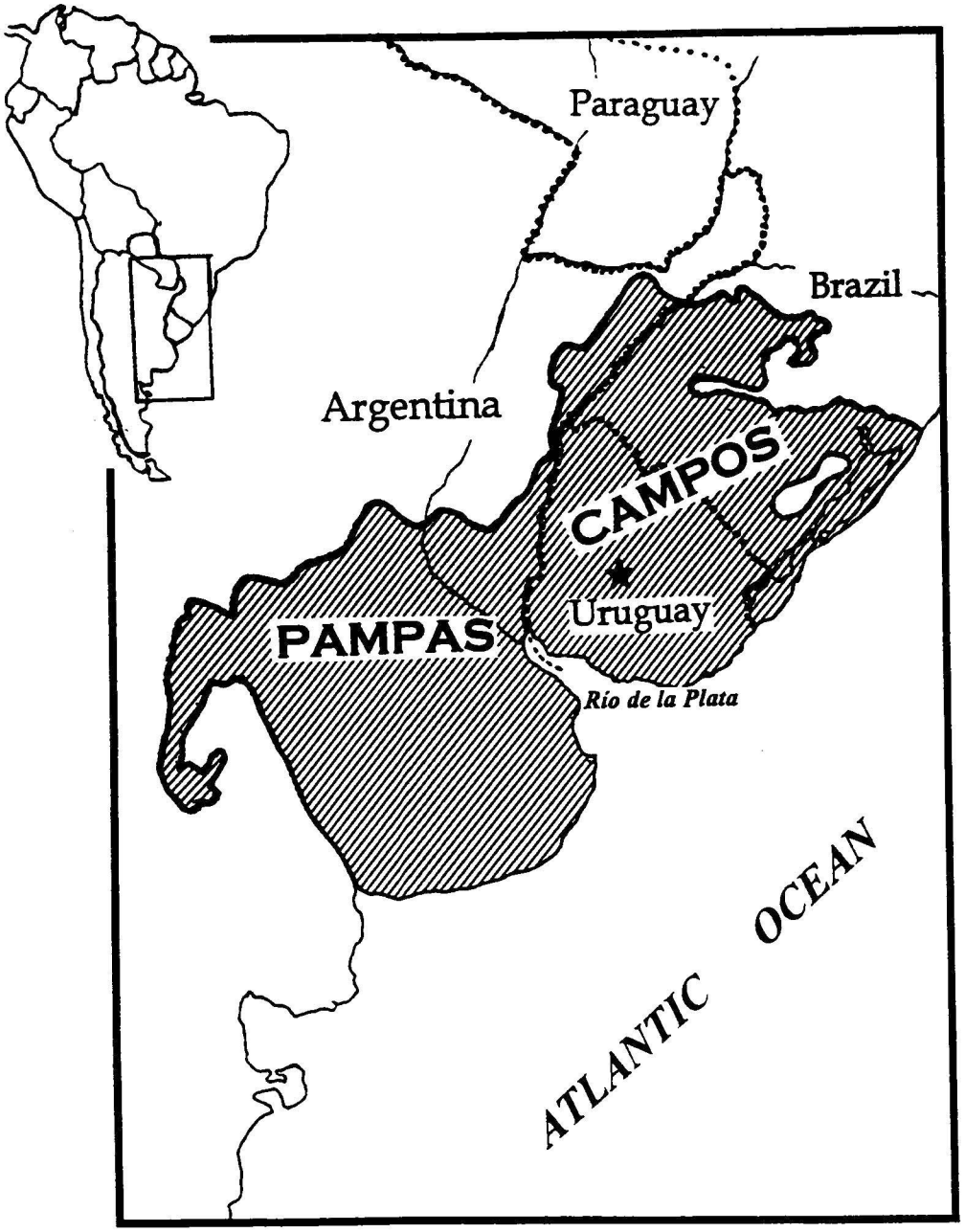

Fig. 1. Outline map of South America (top left corner) showing the Río de la Plata grasslands (inset). The enlargement shows the area defined as the Río de la Plata grasslands (▨), the countries borders (- - ·) and the location in Uruguay where the studies were performed (★).

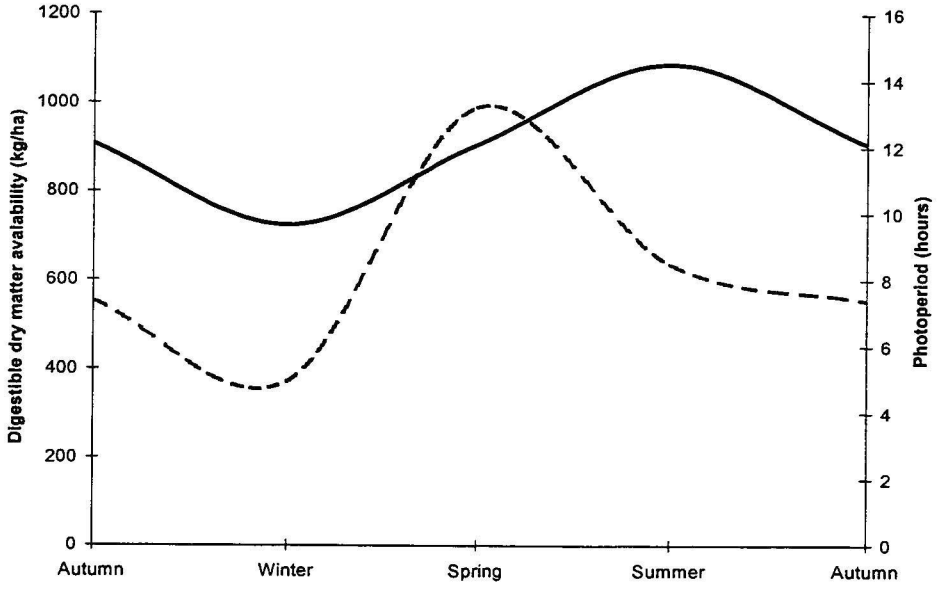

Fig. 2. Annual changes in photoperiod (32°45'SL, full line) and in pasture availability of digestible dry matter (interrupted line) in basaltic soils of Uruguay.

Uruguay

Uruguay is located by the Atlantic coast of South America, between 30° and 35° SL, in the Río de la Plata grassland region (Fig. 1). Uruguayan climate has been classified as humid sub-tropical, according to Köppen's classification as described in Ahrens (1991). Mean temperatures in central Uruguay range from 26°C in January (warmest month) to 12° C in July (coldest month) with clear changes in photoperiod (Fig. 2). Summers are hot and relatively dry but with irregular heavy storms, autumns are mild, winters and springs are humid and windy. There is an average of 40 frosty winter days per year. Annual rainfall is moderate (1,100-1,300 mm) but irregular (with risk of both droughts and floods, Soriano, 1992). About half of the Uruguayan agricultural land (17 million hectares) is classified as pastoral, due to low soil depth, hilly topography and high erosion risk. Some other 11 % (1.9 million hectares) is considered pastoral-agricultural but only patches (approx. 25%) are arable (Cayssials and Alvarez, 1983). During the past 40 years, animal husbandry has been practised on an average of 86% of the agricultural land in Uruguay. Extensive livestock husbandry occupies 78% of Uruguayan land surface. Extensive grazing systems on native pastures are most often used (Berretta et al., 1994), with sheep and cattle grazing together and competing for the same forage resources. In basaltic soils, where most sheep husbandry is held, pastures normally make a peak in dry matter production in spring, and a valley in winter (Berretta et al., 1994) (Fig. 2).

Corriedale sheep

The Corriedale breed originated in New Zealand in 1880-1910 from crossing Lincoln (30%) and Leicester (20%) rams with Merino ewes (50%). It is a dual-purpose (wool and mutton) breed. These medium-wool sheep are well adapted to subtropical-subhumid grasslands. They mature relatively early and have good mutton conformation (Briggs, 1970). Corriedale's wool growth is seasonal, with a maximum in summer and a minimum in winter (Gambetta et al., 1992). The breeding season spans traditionally from late summer to the end of autumn (February-June), with a peak in March-May. The national lambing rate averages 85% with a 72% weaning rate. On average, since sheep raised in extensive systems produce less wool and meat than in intensive systems, the level of exploitation per hectare is far from the potential maximum for Uruguayan Corriedales. Animals suffer frequent winter weight losses, sometimes very pronounced, with negative effects on female reproductive performance and on the level of meat extraction from these systems (Berretta et al., 1994).

Most of the Corriedale world stock (70%) is found within the southern South American region, with Uruguay accounting for 35% of the world's breed population (Azzarini and Cardellino, 1984). Furthermore, around 70% of sheep in southern South America are Corriedale.

Introduction to the studies

Seasonal variations in testis stereology (the discipline that infers three dimensional properties of objects observed two-dimensionally) exist in rams of several breeds (Ortavant, 1959; Hochereau-de Reviers et al., 1976; 1987). Very little information exists for Corriedale rams regarding this subject, information essential to understand photoperiod-nutrition interactions.

The degree of sensitivity of ram testicular form and function to respond to photoperiodical and nutritional cues varies greatly according to the combination of sheep breed and location studied. Corriedale rams in Uruguay show moderate seasonal variations in testicular morphology (Gastel et al., 1995). To what extent feeding management influences testicular parameters in Corriedale rams under Uruguayan pastoral conditions is yet to be determined.

Photoperiod-induced seasonal changes in Merino rams can be overrun by nutritional changes (Martin et al., 1990). On the contrary, this is not the case with highly seasonal breeds. In view of the mixed genetic origin of Corriedales, whether nutritional improvement would affect their testicular size after it has peaked in autumn, is to be disclosed. The results can be used to implement eventual changes in breeding strategies in the region.

Puberty is delayed in Corriedale rams reared under extensive conditions in the Río de la Plata grasslands (Castrillejo et al. 1995) when compared to better-fed lambs. Corriedale wethers keep on growing until three years of age under these extensive conditions (Rodriguez, 1990). Since Sertoli cells stop dividing before puberty is achieved, nutritional effects acting during early life -within the range of what can be applied under extensive management conditions- might affect sperm production capacity in adulthood, by influencing Sertoli cell numbers. Feeding resources are scarce in extensive systems, and sheep are frequently fed sub-optimally. To help achieve a more efficient use of feed in extensive systems, studies of the effects of improved feeding i.e. improved pastures and grain supplementation as compared to native pasture are to be undertaken. These studies should include determinations of changes in testicular parameters and their amplitude and duration, including morphometric traits. In order to achieve this, "windows" of improved nutrition can be studied at different periods of early life.

Aims of the study

The general aim of this thesis was to study the influence of different grazing regimes on testicular morphology and function of Corriedale rams under extensive management conditions, particularly on seasonal changes and testis development. The specific aims were to determine:

- Differences in testicular morphology when young adult rams were raised feeding natural or improved pastures during one year,

- the influence of grazing improved pastures plus grain supplementation on the degree of seasonal variation in testicular morphometry, from autumn to winter, in young rams,

- the effects of long-lasting differential grazing regimes during pre- and post-puberty on the testicular morphology and development of young adult rams and,

- the impact of improved feeding during foetal and early post-natal life on the testicular morphology of ram lambs.

Materials and Methods

Location

The experiments were carried out at "Cabaña El Recreo", a Corriedale nucleus farm located near Carlos Reyles, Durazno, Uruguay (32°45'SL). Daylight at the location varied from about 14h 30 min in December (summer) to about 9h 40 min in June (winter). The farm is located in a basaltic soil region with moderately deep soils. The native pasture is made up of perennial warm-season graminae, perennial cool-season graminae, annual cool-season grasses, native leguminosae and non-graminae.

Animals

Experimental animals were Corriedale male sheep, all born from the same family line at the farm, and of varying age: young adult (**Paper I**: 15-30 months of age, **Paper II**: 18-23 months of age); pre- and post-pubertal period to adult (**Paper III**: 1-30 months of age); and foetal to pre-pubertal periods (**Paper IV**: mid-gestation to 99 days of age).

Experimental Design

Different grazing/feeding strategies were applied to Corriedale male sheep during varying life periods. **Paper I** studied 12 young adult rams grazing improved pasture compared with 12 control rams grazing native pasture over a one-year period (castration/histology studies, clinical recordings and testosterone measurements done every season). **Paper II** studied 20 rams from autumn (March) to winter (August), grazing either native or improved pastures plus 1 kg of grain supplement/ram/day. Castration/histology studies were done in autumn (March, autumn control group, n=6, native pasture) and in winter (August, winter control group, n=7, native pasture; treated group, n=7, improved pasture plus grain supplement). Clinical recordings and testosterone measurements were performed monthly. **Paper III** studied 41 rams from 1 to 30 months of age, grazing either native or improved pastures (groups H and L, respectively; 1-8 months of age, Period 1). By moving half the animals to the opposite treatment, groups HH, HL, LH and LL were created (8-18 months of age, Period 2). Finally, to test whether the changes elicited in testes morphology were present one year after having stopped the differential treatment, all remaining rams from all groups shared the same grazing regime (18-30 months of age, Period 3). Clinical data were recorded every other month. Castration/histology studies in half the animals from each group were done at 18 months (end of differential treatment) and 30 months of age. **Paper IV** studied 14 ewe-lamb units grazing improved pasture and supplemented with grain from mid-pregnancy to pre-pubertal life as compared with 12 ewe-lamb units grazing native pastures (half of lambs castrated for histology at 99 days of age). Clinical and endocrinological studies were performed with varying frequency from birth to castration.

Methods

Recordings of clinical parameters

Total body weight **(Papers I-IV)** was recorded early in the morning with the same scale and always by the same operator. Fleece weight was recorded after shearing. Total body weight was corrected for fleece weight to calculate live weight **(Papers I-III)**. Scrotal circumference **(Papers I-III)** was recorded, always by the same operator, using a flexible tape, at the widest scrotal diameter. Semen was collected **(Paper I)** by electroejaculation, and samples fixed in buffered formalin solution or prepared as air-dried smears for morphological examination. Sperm morphology was examined with light microscopy on carbol-fuchsin stained smears (Williams, 1920), and on unstained wet smears with phase contrast optics at x1000.

Blood sampling and hormone assays

Single blood samples were withdrawn from each ram, also early in the morning, by jugular venipuncture every three months **(Paper I)**, monthly **(Paper II)**, and at 45, 75 and 99 days of lamb age **(Paper IV)**. Blood was stored **(Paper I)** in heparinised tubes (145 USP sodium heparin) and immediately placed on ice. The blood plasma, harvested after centrifugation, was stored at -20°C until analyzed. In **Papers II** and **IV**, the blood was left to clot at ambient temperature, whereupon the serum was harvested and stored at -20°C until analysed. The blood plasma/serum samples were analysed by RIA for testosterone **(Papers I, II** and **IV)** and FSH **(Paper IV)**.

Testicular and epididymal weight and volume recordings

After castration, testes and ductus epididymides were examined macroscopically **(Papers I-IV)**. Testes weight was recorded using a mechanical balance to the nearest 0.1 g **(Papers II-IV)**. Testes volume was recorded by immersion in normal saline in a graduated vessel with a precision of either 10-ml units **(Papers II** and **III)** or 1-ml units **(Paper IV)**. Ductus epidydimides were dissected free and weighed **(Paper IV)**.

Tissue sampling and fixation

Tissue samples (1 cm^3) from the testis (proximal, medial and distal testis locations) and epididymis (caput [region 1], corpus [region 5] and cauda [region 6], Nicander, 1958) as well as medial pieces from the seminal vesicle were collected **(Paper I)**. Samples were immersed in Bouin's solution for 24 hours, paraffin embedded and stained with hematoxylin and eosin (HE). In other experiments **(Papers II-IV)**, one testis per animal was fixed by vascular perfusion. Samples (1 cm thick for paraffin embedding, 1 mm thick for resin embedding) were removed from proximal, medial and distal testis locations and stored in the fixative solution until further processed. Thick sections obtained from the larger samples were HE-stained. The smaller samples were dehydrated and embedded in metacrylate. Because the inclusion procedure provokes some

cell/tissue shrinkage (Wrobel et al., 1995), one sample was measured before and after inclusion. The resulting shrinkage percentage was applied to all absolute calculations made thereafter. Sections were cut and stained with HE.

Stereology

The diameter of the seminiferous tubules, or of testicular (sex) cords in some cases, **(Paper IV)** was measured in thick paraffin sections **(Papers I-IV)**, in two perpendicular diameters from either 50 **(Paper I)** or 30 **(Papers II-IV)** randomly chosen cross-sections from each of the three regions (proximal, medial and distal) per testis. Measurements were performed at 100x in an Olympus BH microscope (Olympus, Japan) with an ocular reticulum (1/100 mm). Stereological studies **(Papers II-IV)** were performed on HE-stained, 2-µm-thick metacrylate sections with an image analysis system (Instrument AB, Solna, Sweden) on images retrieved with a Nikon Microphot-FXA light microscope (Nikon, Japan). The volume densities of several histological structures were determined by point counting (Weibel, 1979; Elias and Hyde, 1980) at a final magnification of 900x. The measured structures were as follows: seminiferous tubules **(Paper III)**; seminiferous tubules, blood vessels, Leydig cells cytoplasm, Leydig cells nuclei and interstitium (without Leydig cells and blood vessels) **(Paper II)**. In **Paper IV**, the seminiferous compartment (corresponding to seminiferous tubules in **Papers II and III**) was further classified into seminiferous epithelium, seminiferous tubule lumen and Sertoli cell nuclei. The volume density of lynph vessels was also determined **(Paper IV)**. The point counting procedure was performed by superimposing a grid of 20 intersecting lines (3,360.44 μm^2 area) over the screen image of every microscopic field studied. The number of intersections on the grid (test points, n= 100) overlying the tissue component of interest was then counted, and the ratio of the number of these points to the total number was considered to be the volume density of that component (Equation 1):

$$V_v = Pn / Pt \qquad (1)$$

where V_v is the volume density of the tissue component of interest, Pn is the number of intersections on the grid overlying the tissue component of interest, and Pt is the total number of points on the grid. Thirty randomly selected fields were examined from each testicular region, i.e. 90 fields (302,439.60 μm^2 and 9,000 test points) per animal **(Paper II)**. In **Papers III and IV**, only 30 fields per testis were examined. Absolute volumes of these parameters **(Papers II-IV)** were calculated by multiplying the volume density of the tissue component of interest times the testicular weight (testicular density was assumed to be 1). The total number of Sertoli cells per testis **(Papers II-IV)** was estimated with Equation 2:

$$\text{Total No. Sertoli cells} = Ns \times (L \div [\text{Section Thickness}]) \qquad (2)$$

where Ns is the mean number of Sertoli cells per transversal section of seminiferous tubule (testicular cord in some cases, **Paper IV**), and L is the total length of the seminiferous tubule. The seminiferous tubules were assumed to be cylindrical, and their lengths were estimated from equations 3 and 4 (Marshall and Plant, 1996):

$$L = V_s \div (\pi \times [D_s \div 2]^2) \quad (3)$$

$$V_s = (V_v \text{ of } ST) \times \text{absolute testicular volume} \quad (4)$$

where V_s is the total volume of the seminiferous tubules, and D_s is the diameter of the seminiferous tubules. The number of Sertoli cells per cross section of seminiferous tubule was multiplied by the total seminiferous tubules length to yield the number of Sertoli cells per testis. Seminiferous parenchyma, containing the seminiferous tubules, occupies most of the testis (May, 1964). However, seminiferous parenchyma was assumed to occupy all the testicular volume, as it is standard in testis stereology (Hochereau-de Reviers et al., 1976; Johnson and Nguyen, 1986). The number of Leydig cells **(Papers II and IV)** was calculated using the Floderus equation as described in Weibel (1979):

$$N_V = N_A/(D+T-2h) \quad (5)$$

where N_V is the numerical density of Leydig cells (number of Leydig cells/unit volume of testis), N_A is the number of Leydig cell nuclei/unit area of testis on the section, D is the mean diameter of Leydig cell nuclei on the section, T is the average section thickness (2 μm) and h is the correction factor for "lost caps" (polar sections of Leydig cell nuclei not large enough to be identified) and was assumed to be D/10 (Christensen and Peacock, 1980). Leydig cell nuclear diameters were measured in 30 nuclei per ram, only if the optical middle of the nucleus, where the diameter was maximal, could be found at some focal level within the section (Christensen and Peacock, 1980). These data were obtained with a 60x-immersion oil objective at a final magnification of 2,700x.

Elongated spermatid counts

The procedure for counting elongated spermatids **(Papers II and III)** was modified from the adaptation of Amman's technique described by Walkden-Brown et al. (1994). The unfixed testis from each animal was dissected free, and its parenchyma was chopped and frozen until further processing. Thereafter, the parenchyma was homogenised in a blender together with 200 ml of detergent solution. The resulting homogenate was further diluted and mixed. An aliquot of this suspension was filtered to remove large debris, and quadruplicate counts of testicular elongated spermatids were made using a Neubauer haemocytometer.

Statistical methods

Data were treated and analysed statistically using the SAS package (Statistical Analysis Systems Institute Inc., 1987: **Paper I**; 1993: **Papers II-IV**). The collected data, including information describing the overall population and the animals selected for morphological examination, were expressed as least square means ± s.e.m. (**Paper I**) or SD (**Papers II-IV**). Hormonal values were $\log_{10}$ transformed (**Papers II and IV**) since most of them (when classified according to treatment group and month) failed to show normal distribution according to Shapiro-Wilk's test. Hormonal numerical values were presented as the anti-logarithms of least-square means of the $\log_{10}$ values (geometric means) with a 95% confidence interval, which appears in parenthesis. Data were analised either by ANOVA (**Papers I-IV**) or by Student's t test (**Paper IV**). Either Pearson correlation coefficients (**Paper I**) or Spearman's rank correlation coefficients (**Paper II**) were studied between selected parameters. Residual Pearson's correlations (**Papers III and IV**) were calculated after correction for the effect of treatment. Correlations between the parameters were calculated after expressing each value as a deviation from the mean of the corresponding treatment group and recording occasion (**Paper IV**).

Results

Grazing management and male seasonality

Seasonality of testis morphology of young adult Corriedale rams grazing either native or improved pastures ***(Paper I)***.

Rams on native pasture lost weight during winter (-24%, $P<0.01$) but regained it as summer approached. In contrast, rams on improved pasture kept live weight fairly constant throughout winter and increased it during the rest of the experiment ($P<0.05$). Scrotal circumference (SC) decreased significantly during winter ($P<0.001$) in both groups, but was lower ($P<0.05$) in rams on native pasture, where SC had yet not returned to its initial value by the end of the study ($P<0.05$). In rams on improved pasture, SC had already returned to its initial value by spring and had increased significantly ($P<0.001$) by the end of the experimental period (summer). Live weight (LW) was significantly correlated with SC at both feeding levels (rams on native pasture, $r= 0.85$, $P= 0.001$; rams on improved pasture, $r= 0.73$, $P<0.01$). Plasma levels of testosterone differed ($P<0.05$) only between autumn levels (rams on improved pasture) and spring levels (both rams on native and on improved pastures). No significant month or feed effects were present. Semen could always be collected from the rams in all seasons and the frequency of spermatozoa with deviant morphology was always low. No seasonal or feeding level effects on semen parameters were found. Spermatogenetic activity was present in all seasons in both groups. The diameter of the seminiferous tubules at both feeding levels decreased significantly during winter ($P<0.01$-0.001 resp.) but increased steadily thereafter, to return to initial values by spring (rams on improved pasture) or summer (rams on native pasture). There were group differences in the diameter of the seminiferous tubules only in autumn and summer ($P<0.05$). This diameter was significantly correlated with SC ($r=0.86$, $P<0.01$ for the rams on native pasture and $r=0.91$, $P<0.001$ for the rams on improved pasture). The seminiferous tubules diameter also appeared to be correlated with live weight, although only in animals on improved pasture ($r=0.67$, $P=0.02$).

The morphology of the ductus epididymides appeared to be normal. The lumen of cauda epididymidis was well-filled with normal-looking spermatozoa in both groups. The height of the epididymal epithelium did not vary significantly over the year in the caput, corpus or cauda regions. The epithelium of the seminal vesicle showed the highest epithelial cell height during autumn, while the lowest cell height was present during winter ($P<0.01$-0.05 resp.) for both groups of rams. The epithelial height of the seminal vesicles was significantly correlated with the seminiferous tubules diameter (rams on native pasture: $r=0.91$, $P<0.01$; rams on improved pasture: $r=0.72$, $P<0.01$) as well as with SC (rams on native pasture: $r=0.65$, $P<0.05$; rams on improved pasture: $r=0.78$, $P<0.01$).

In summary, young adult Corriedale rams grazing improved pasture showed a smaller winter testicular regression and an earlier testicular recovery in spring than rams grazing native pasture.

Autumn-winter seasonal variations in testis morphology in young adult Corriedale rams fed either native or improved pastures plus grain supplement ***(Paper II)***.

Mean LW increased significantly in grain-supplemented animals ($P<0.01$) but not in rams on native pasture. However, LW did not differ significantly between groups by the end of the experiment. Therefore, a regression analysis for histological traits was run with LW as a covariant, showing that no significant LW-effect was present for any of the stereological variables analysed. Mean SC decreased from autumn to winter in both groups ($P<0.01$-0.05 resp.), with lowest values in winter. The values of SC did not differ between the two groups on any of the sampling occasions. Serum testosterone concentrations decreased during the experiment in both groups ($P<0.05$), with highest levels in autumn and lowest in winter. Testicular weight and volume decreased in both groups from autumn to winter ($P<0.001$). Testicular weight in winter was higher in grain-supplemented rams than in rams on native pasture ($P<0.05$), but there was no corresponding difference in testicular volume. In winter, right testis weight and right testicular parenchymal weight expressed as percentages of LW were 0.21% and 0.20% for rams on native pasture, and 0.24% and 0.22% for grain-supplemented rams, respectively. The diameter of the seminiferous tubules and the number of elongated spermatids per Sertoli cell in winter were higher in supplemented rams ($P<0.05$). Conversely, the number of Sertoli cells per ml of testis was higher ($P<0.05$) in control rams. However, all other stereological parameters did not differ between supplemented and control rams. Testicular volume was correlated with the diameter of the seminiferous tubules ($r=0.55$, $P=0.012$). The amount of elongated spermatids per testis was correlated with the number of Sertoli cells per testis ($r=0.69$, $P=0.001$) and with the volume density of seminiferous tubules ($r=0.49$, $P=0.032$).

In conclusion, there was a moderate, but clear reduction in the spermatogenetic tissue volume and activity and a less marked reduction in interstitial tissue in the testes of Corriedale rams reared on native pastures between autumn and winter. The results further indicate that grazing improved pasture with additional grain supplementation as performed, were unable to reverse the winter reductions in testis size and spermatogenesis.

Grazing management and testicular development

Testis growth and morphology at 18 and 30 months of age in Corriedale rams grazing either native or improved pastures during pre- and post-pubertal life ***(Paper III)***.

Period 1 (1-8 months of age): LW increased significantly during the study

period ($P<0.05$). Both LW and SC were significantly higher ($P<0.05$-0.01 resp.) in rams grazing improved pasture.

Period 2 (8-18 months of age): LW increased significantly ($P<0.05$) in all groups, and differed among groups ($P<0.05$) by the end of this period, ranking from highest to lowest as follows: groups HH, LH, HL and LL (for group description see Materials and Methods, Experimental Design section). Scrotal circumference followed a similar trend but group LH equalised group HH during the second half of Period 2, while group HL caught up with group LH by the end of Period 2. Neither testicular weight, volume nor stereological parameters (diameter, volume and volume density of seminiferous tubules, number of Sertoli cells per testis, elongated spermatids per testis) differed among treatment groups by the end of Period 2.

Period 3 (18-30 months of age): LW differences diminished during this period. Groups HL and LL showed no differences, and group LH caught up with group HH during the second half of the period. Scrotal circumference among groups was not different during most of Period 3. There were no differences in testicular weight, volume nor stereological parameters by the end of Period 3. However, when comparing values at the end of Periods 2 and 3, significant increases were recorded for testis weight and volume ($P<0.05$) in groups LL and LH, for the diameter of the seminiferous tubules ($P<0.05$) in groups LL and HL, for the volume of the seminiferous tubules ($P<0.05$) in group LL, and for the number of elongated spermatids per testis in all groups. Both LW and SC were correlated (experimental animals grouped by castration year) significantly ($P<0.05$ - $P<0.01$) irrespective of when castration was performed (i.e. at 18 or 30 months of age). However, neither testis weight, seminiferous tubules volume nor Sertoli cell numbers per testis correlated significantly with LW (n.s.) once the mean effect of treatments was put aside. Elongated spermatid numbers per testis correlated with LW only at 30 months of age ($P<0.05$). Scrotal circumference also correlated with testicular weight ($P<0.001$), volume of seminiferous tubules ($P<0.001$) and elongated spermatid counts ($P<0.01$ at 18 months, $P<0.001$ at 30 months of age) at both ages of castration, but not with Sertoli cell numbers per testis (n.s.). Testicular weight correlated with the volume of the seminiferous tubules ($P<0.001$) and elongated spermatid counts ($P<0.001$ at 18 months of age, $P<0.05$ at 30 months of age) on both castration ages. Sertoli cell numbers per testis correlated with testicular weight at 30 months of age ($P<0.001$) but not at 18 months of age (n.s.). Seminiferous tubules volume correlated with Sertoli cell numbers ($P<0.05$ and $P<0.001$ resp.) and elongated spermatid counts ($P<0.001$ and $P<0.05$ resp.) at 18 months and 30 months of age.

The results show that grazing improved pasture during pre- and post-pubertal periods of life induced differential rates, albeit short-lived, of testis development in Corriedale rams.

Influence of grazing management of the ewe-lamb unit on testis morphology in Corriedale lambs ***(Paper IV)***.

Mean body weight (BW) increased significantly ($P<0.001$) between any two consecutive sampling dates both in treated (better fed) and in control (poorly fed, controls) animals. Moreover, BW differed between treated and control animals at all recording occasions. Daily weight gain was higher ($P<0.001$) in treated than in control lambs. Serum concentrations of testosterone never differed significantly, neither between different recording occasions within the same group nor between groups at the same recording occasion. FSH levels tended (n.s.) to differ between groups at 45 days of age, but not later. Serum concentrations of FSH declined during the experiment in both groups of animals. Testicular and epididymal weight and volume were smaller in control than in treated animals ($P<0.01$). The morphology of the testicular parenchyma was very different in treated lambs as compared with controls. The diameter of the testicular cords/seminiferous tubules differed between treated and control groups ($P<0.01$). Volume densities of the seminiferous epithelium ($P<0.05$), their tubular lumen ($P<0.05$), Sertoli cell nuclei ($P<0.01$) and seminiferous compartment ($P<0.05$) were significantly higher in treated compared with control animals. Volumes of the seminiferous epithelium ($P<0.001$), Sertoli cells nuclei ($P<0.01$) and total seminiferous compartment ($P<0.001$) were significantly higher in treated animals compared to controls. Volume densities of blood and lymph vessels and of Leydig cells cytoplasm and nuclei did not differ. Volumes of blood and lymph vessels were not different either, but volumes of Leydig cells nuclei and cytoplasm were significantly higher ($P<0.001$) in treated than in control animals. Sertoli cell numbers per testis tended to be higher ($P<0.07$, Student's t-test) in treated than in control animals, while Sertoli cell numbers per ml of testis were higher ($P<0.05$) in control lambs as were Leydig cell numbers per testis ($P<0.001$). Correlations between BW, testicular weight and stereological variables showed that seminiferous tubules/testicular cords volume correlated significantly with seminiferous tubules lumen volume density ($r=0.82$, $P<0.01$) and volume ($r=0.89$, $P<0.001$). Body weight, however, did not correlate significantly with most testicular parameters.

These results indicate that the earlier pubertal development occurring in extensively reared Corriedale ram lambs grazing improved pastures and being supplemented with grain was accompanied by a tendency to have higher Sertoli cell numbers.

General Discussion

Methodological considerations

Quantitative histology

Morphology often helps us to understand physiological phenomena, as a result of the link between form and function. Although this link might be evident at the macroscopical level (testis size or scrotal circumference as an indicator of testicular activity), in the present thesis the form-function relationship was mainly studied at the histological level. While major morphological changes can easily be described by qualitative (subjective) evaluations, the study of subtler effects on testicular histology requires the quantification of cellular parameters as attempted in this thesis. The stereological analyses were complemented with clinical recordings and restricted endocrinological analyses in order to broaden the scope of information obtained and to facilitate its comparison with studies run using other breeds and conditions.

Pen vs paddock studies

The literature reports abundantly on photoperiod-nutrition interactions and nutritional influences on pubertal testicular development in rams. However, when this thesis work began, there was practically no information concerning rams of the Corriedale breed, the majoritarian sheep breed in the South American region. As well, most published information referred to nutritionally controlled ("penned") experiments. This type of experiments requires expensive installations and feeding controls and provides very useful information. However, at places where most sheep breeding is done on extensive systems, on-farm field experiments would provide results that can be easily transferred to practice. Although this approach may not gather all information regarding what the rams really consume, and diminish our control over feeding quality and quantity, much is gained regarding the link of the results obtained to real-life production systems in the region, an obvious advantage under South American conditions. The experiments performed in this thesis were very similar to the systems of sheep production that are run under the Rio de la Plata region conditions. Because a background information about penned and paddock experiments is available for other places and breeds, it thus appeared very reasonable to run the experiments on-farm.

Grazing management and ram seasonality

In the present thesis, the testicular morphometry of Corriedale rams was assessed throughout different seasons, confirming the occurrence of moderate seasonal variations in Corriedale rams reproductive activity in extensive management systems (Gastel et al., 1995). Changes during autumn and winter in scrotal circumference (SC), testicular weight and volume, as well as in diameter, volume density and absolute volume of the seminiferous tubules, and the

numbers of testicular elongated spermatids per testis and per Sertoli cell (thus monitoring testicular sperm production), all indicate that there was a reduction in spermatogenetic activity between autumn and winter. Seasonal changes also occurred in testosterone release (as decreasing serum testosterone levels in control rams were seen between autumn and winter)(**Paper II**). Neither Leydig cell nuclear volume nor Leydig cell numbers showed any seasonal change, but Leydig cell cytoplasmic volume tended to decrease between autumn and winter, resembling the trend found in testosterone levels. Ile-de-France rams (a highly seasonal breed) show seasonal variations in both the seminiferous tubules volume and of interstitial tissue volume (Hochereau-de Reviers et al., 1976) between autumn and spring. It appears interesting that the seasonal variations in testicular interstitium parameters seen in Corriedale rams were less marked than the pattern described for Ile-de-France rams. The seasonality shown by the testicular morphometry parameters in Corriedale rams suggests that the testis size changes are linked mostly to variations in spermatogenetic tissue rather than to changes in the interstitium.

The finding that Sertoli cell numbers were higher in control rams castrated in autumn than in those castrated in winter was unexpected (**Paper II**). It suggests that some Sertoli cells died during winter. Sertoli cell numbers have been reported to decrease with age in horses (Johnson and Thompson, 1983), men (Johnson et al., 1984), rats (Wang et al., 1993), hamsters (Horn et al., 1996) and donkeys (Nipken and Wrobel, 1997). Interestingly, in donkeys this decrement was evident from puberty and onwards (Nipken and Wrobel, 1997). Hochereau-de Reviers et al. (1985) found no difference in Sertoli cell numbers between Soay rams subjected to short or to long photoperiods. The difference between Soay and Corriedale rams could have been due to differences in management (Soay rams were housed in a controlled environment indoors) or methodological procedures used. However, since Sertoli cells do not divide after puberty, the difference in Sertoli cell numbers found between autumn and the subsequent winter is probably related to the aging process, as reported in other mammalian species (see references cited above). The variation in Sertoli cell numbers seen hereby must be confirmed in follow-up, long-lasting studies of a larger animal population.

A higher feeding level moderated the winter decrement in testis size and then hastened testis size recovery (**Paper I**). The variations registered in seminiferous tubular diameter and in SC reflected variations in the amount of spermatogenetic tissue present in these testes, and strongly suggest the existence of seasonal changes in sperm production in Corriedale rams. The percentage of sperm abnormalities showed no important variation throughout the year. The earlier testis size recovery occurring in spring due to improved nutrition suggests that Corriedale rams are less seasonal than Scottish Blackface and Finnish Landrace x Dorset rams, which respond to nutritional cues only in autumn (Alkass et al., 1982).

The results (**Paper I**) confirm the existence of seasonal variations in testes morphology and point out that photoperiod is an important cue for such variations. These results also show that improvements in nutritional availability and quality, made within the possibilities of an extensive feeding system, could diminish the degree of testicular regression and significantly hasten the start of testicular recrudescence in Corriedale rams.

The experiment reported in **Paper II** was conducted on an extensive rearing system, and climatic factors could have affected the results. The weather that prevailed during autumn (combination of high rainfall and relatively mild temperatures) rendered the conditions on native pastures very favourable, even in winter, as reflected in the live weight (LW) gain in control animals. These grazing conditions could explain why LW was not different between rams on native pasture or those grazing improved pasture plus grain supplements. However, unlike what occurs in Merino rams (Murray et al., 1991), once the seasonal reduction in SC of Corriedale rams had begun, improved nutrition could not reverse the trend. The treatment applied could not reverse the normal seasonal trend in serum levels of testosterone reported for Corriedale rams (Pérez et al., 1997) despite the steady increase in LW that occurred in grain-supplemented rams. The treatment could only postpone the decrease in testosterone levels in late autumn, when rams on improved pastures had higher levels than those on native pasture.

For animals castrated in winter, some parameters (SC, testis volume, volume density and absolute volume of seminiferous tubules, testicular interstitium volume, number of elongated spermatids, number of Sertoli cells per testis) did not differ between control and treated rams (**Paper II**). However, testis weight, parenchymal weight, seminiferous tubules diameter, were higher in treated than in control rams. These changes in stereological parameters suggest that the treatment had a mild effect on spermatogenetic activity. The lack of difference in testicular interstitium volume between treated and control rams in winter was not surprising (**Paper II**). The same occurs in Merino rams, where increments in testis size due to improved nutrition can be attributed exclusively to increases in seminiferous tubules' absolute volume; i.e. there is no accompanying increment in interstitial tissue volume (Martin et al., 1994).

Sertoli cell numbers did not differ with different feeding levels in winter, indicating that the treatment applied in this study did not help to reduce the extent to which the winter Sertoli cell population decreased from the previous autumn's levels (**Paper II**). Testicular elongated spermatid numbers were lower in winter-castrated grain-supplemented rams than in autumn-castrated rams on native pasture, indicating again that the treatment applied was unable to reverse the winter seasonal reduction in spermatogenetic activity. Moreover, the lack of significant difference in the number of elongated spermatids between winter-castrated rams on native pasture or grain-supplemented rams is probably

explained by the very good conditions that the rams had on native pastures during winter.

In summary, the effects of feeding regimes on the seasonal testicular changes of Corriedale rams in extensive grazing management systems were studied by having rams grazed either native or improved pasture along one year **(Paper I)** and further, by feeding rams either native or improved pastures plus grain supplement from autumn to winter **(Paper II)**. In both experiments, all parameters reflecting spermatogenetic activity decreased moderately from autumn to winter, regardless of LW evolution: LW either diminished (control rams, **Paper I**), did not change (rams on improved pasture, **Paper I** and rams on native pasture, **Paper II**) or increased (rams on improved pasture and supplemented, **Paper II**). The limited effects of improved nutrition on the decrease in testicular size of Corriedale rams suggest that Corriedale rams are intermediate in seasonality between Merino rams (whose testicular cycle is driven primarily by feed variations that can overrun photoperiodic influences) and those of breeds such as Ile-de-France or Suffolk (that did not show differences in testis size related to differences in diet during the non-breeding season) (Lindsay et al., 1984, Hötzel et al., 1994). However, Corriedale rams seem to be closer to highly seasonal breeds (where there is no response to nutrition during the testicular regression period) than to less seasonal breeds (where nutrition can override photoperiodic influences any time of the year).

Grazing management and testicular development in Corriedale rams

Increases in LW and SC (**Paper III**) confirmed for Corriedales what is already known for other sheep breeds, namely, that better feeding in early ages (1-8 months) hastens testicular development. A similar trend was found at 8-18 months of age, which implies that rams on improved pasture developed their testes faster than those on native pasture. It is interesting to note that by the end of this period, the percentual constitution of the testicular parenchyma was already of adult type and there were no differences in this respect between treatment groups. During most of the period from 18-30 months of age, when all rams were on native pasture, SC and stereological parameters did not differ between groups. Therefore, the influence of nutritional history on testicular size and activity during pre- and post-pubertal life is short-lived in Corriedale rams. Sertoli cells numbers did not differ between groups at any castration period, because Sertoli cells stop dividing at 40-60 days, before the major interval of the differential treatment period occurred.

In Merinos it has been found that nutrition hastens testicular development during and after puberty (Sutama and Edey, 1985; 1986). However, these differences disappeared soon after nutritional differences ended. In this regard, testicular

development and its interaction with nutrition in extensive grazing systems seems to be similar in both Corriedale and Merino rams. The fact that testicular stereology was not affected by feeding management might also be true in other breeds of sheep.

Pubertal development was hastened in ram lambs by the use of improved pasture and grain supplementation (**Paper IV**). Body weight changes indicated that the treatment imposed on the ewe/lamb unit had a clear effect on lamb growth. Increased body weight and daily weight gain in better-fed animals was not accompanied by higher testosterone levels, but sampling frequency might explain why differences were not detected. Declining FSH levels confirm the decline in FSH concentrations during the pubertal period reported for Merino x Romney lambs (Isaacs et al., 1994). Testes and epididymides weight, testes volume and most stereological parameters indicate that puberty occurred earlier in treated than in control animals. Sertoli cell numbers tended to be higher in treated than in control lambs. One possible explanation for this lack of statistical significance is the relatively large variance in the treated group, which might be explained by differences in individual responsiveness to the treatment applied. The experimental error due to methodological variability in Sertoli cell counts might also be an important factor. The tendency to a higher Sertoli cell population found in treated lambs may have been caused by their higher FSH levels. However, the results did not confirm that this was the case. Probably, most of the period with differences in FSH levels had occurred at younger ages, before blood sampling began. The tendency to higher Sertoli cell numbers per testis in supplemented lambs suggests there can be long lasting effects of nutritional treatments during this period of life.

The present finding is important for husbandry strategies in extensive production systems because it suggests that rams reared under extensive conditions are far from reaching their maximum genetic potential for sperm production. This could be alleviated, at least partially, by grain supplementation from foetal to pre-pubertal life.

To our knowledge, comparable experiments have not been performed in any other sheep breed. Undernourished Romanov x Limousin lambs had lower Sertoli cell numbers than normally fed lambs (Brongniart et al., 1985). The present experiment was run under extensive grazing system conditions, where lambs are usually subjected to nutritional constraints, but such lambs should not be considered as undernourished, considering the mean daily gain weight of 194.9 grams per day registered in the control group. It may be that the nutritional constraints imposed by grazing native pasture were not enough to provoke a statistically significant difference when compared with animals on improved pasture grazing and supplemented with grain.

In summary, the treatments applied during the pre- and post-pubertal life of Corriedale rams determined different rates of testicular development before and after puberty, where the rams kept on native pasture during the entire period, showed the slowest testicular development rates. However, these differences in testicular development were not present one year after the different treatments had ended. There were no important differences in Sertoli cell numbers between groups. Different grazing managements during this period of life resulted in short-lived differences in testicular development in Corriedale rams but did not seem to influence testicular form and function later in life. On the other hand, the earlier pubertal development elicited in Corriedale ram lambs raised on extensive management systems, when grazing improved pasture and supplemented with grain during intrauterine and post-natal life was accompanied by a tendency to higher Sertoli cell numbers. The question of whether this tendency can be maintained over time, and for how long, arises. Sertoli cells do not normally proliferate after puberty, but they can die; thus, differences established before puberty do not necessarily persist throughout life.

General Conclusions

Based on the results of the present studies, the following conclusions can be drawn:

- There was a moderate, but clear reduction in the spermatogenetic tissue volume and activity and a less marked reduction in interstitial tissue in the testes of extensively reared Corriedale rams between autumn and winter.

- Young Corriedale rams grazing improved pasture showed a smaller winter testicular regression and an earlier testicular recrudescence in spring than rams grazing native pasture.

- Grazing improved pasture with grain supplementation could not reverse the observed winter reductions in testis size and spermatogenesis and alleviated these reductions only slightly as compared to the control group.

- Grazing improved pasture during the pre- and post-pubertal periods of life resulted in different rates of testis development, of short-lived nature, without showing important differences in Sertoli cell numbers between groups.

- Corriedale ram lambs raised on extensive systems had an earlier pubertal development when they were offered improved pasture and grain supplementation. This was accompanied by a tendency to have higher numbers of Sertoli cells.

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